Text S1: Supplemental Methods for Transposon Library Construction and Sequencing

The pSAM_Cam vector used to generate the transposon library was constructed as follows, using primers listed in Table S2: The chloramphenicol acetyl transferase gene from pKD3 (1) was amplified via PCR that added flanking MluI restriction sites. This PCR fragment and plasmid pSAM_Ec (2) were digested with MluI and ligated to exchange ampicillin resistance for chloramphenicol resistance. Plasmid construction was verified by restriction digest and PCR, and the plasmid was propagated in *E. coli* S17 λpir.

The transposon mutant library was created by mating mid-log cultures of *E. coli* S17 λpir carrying the pSAM_Cam donor strain with *K. pneumoniae* KPPR1in a 2:1 ratio. The resulting mixture was washed with PBS, resuspended in LB broth, spread on 0.45 μm filter disks (Millipore, Billerica, MA) on LB agar plates, and incubated at 37°C for 2 hrs. To induce expression of the transposase from the P_{lac} promoter, filters were transferred to agar plates with 250 μM IPTG (Invitrogen, Carlsbad, CA) and incubated at 37°C for 2.5 hrs. Bacteria were rinsed off the filters into LB broth and plated on LB agar with rifampin and kanamycin to select for *K. pneumoniae* with transposon insertions and enumerate transposon mutant CFU. The resulting transposon mutant library was scraped off agar plates, resuspended in LB with 25% glycerol (final concentration) and stored at -80°C as a single pool in 1 mL aliquots.

To verify that conjugation and induction of transposase on pSAM_Ec produced transposon mutants with the loss of the vector, a portion of the mated bacteria were also patched on agar with chloramphenicol (n=100) to determine the frequency of vector integration and colony PCR from the original colony was performed using primers homologous to the transposon and vector (n=38; Table S2). Verification of a single, random transposon insertion per mutant was accomplished by performing a Southern blot (3, 4) on EcoRI digested genomic DNA using a digoxigenin labeled probe against the transposon (n=38).

Based on a genome size of 5191 predicted genes in a 5.3 MB genome, 24,000 random transposon mutants should represent 99% probability of an insertion in each KPPR1 gene (GenBank ID: CP009208) (5). Accordingly, an inoculum of 1.1 x 10^5 CFU provides a 99% probability that each mutant is represented at least once in the lung. Pilot experiments with varying inocula indicated a proportionate bottleneck such that 10-20% of the inoculum reaches the lung (data not shown). To compensate for this bottleneck and ensure sufficient coverage, 1.4 x 10^6 CFU of ~25,000 transposon mutants were used to inoculate mice.

Following infection, bacterial colonies cultured from lung tissue were scraped from agar plates generating an output pool of mutants. Genomic DNA was harvested from both input and output mutant pools with DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) according to the protocol for Gram-negative bacteria. Next, genomic DNA adjacent to transposon insertion sites was

amplified and adapters for Illumina sequencing were ligated as described (6, 7). Transposon junction DNA fragments were subjected to single end 50 rapid run sequencing on an Illumina HiSeq2500 Instrument (Illumina, San Diego). The resulting reads were filtered, mapped and normalized as described (6).

Supplemental References

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